

# Evolutionary dynamics of declining melanism in the peppered moth in The Netherlands

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Populations of *Biston betularia* in the region of The Netherlands around Leiden and Rotterdam were resampled. A comparison of three sets of data for 1969–1973, 1988 and 1999 enabled a further examination of declines in melanism. Unlike parallel changes for the black *carbonaria* form of this species in urban regions of Britain, those in The Netherlands involve substantial changes in frequencies of at least two of the intermediate *insularia* morphs as well as an increase in the non-melanic *typica* morph. The darkest of the three *insularia* morphs has shown a transitory pulse of increased frequency in The Netherlands. The dynamics are discussed in relation to the history of air pollution and to straightforward predictions about selection.

**Keywords:** industrial melanism; *Biston betularia*; *carbonaria* morph; natural selection; adaptation; evolution

## 1. INTRODUCTION

The evolution of industrial melanism in the peppered moth (*Biston betularia*) has been thoroughly documented in Britain. Steward (1977) was able to use a combination of the literature and museum collections in tracking the earlier spread of the black *carbonaria* morph from its initial sightings in the mid-19th century. This followed Haldane's (1924) well-known calculation of the fitness advantage necessary for accounting for the spread of *carbonaria*. Rich data sets collected by other researchers have documented the spatial correlation between melanic frequencies and levels of air pollutants during the mid-20th century when allele frequencies appeared to be more or less stable (see the reviews by Kettlewell 1973; Lees 1981; Majerus 1998; Cook 2000). Kettlewell (1955, 1973), Bishop (1972) and Bishop *et al.* (1978) obtained estimates of the relative fitnesses of melanic and non-melanic morphs and of migration rates. In addition, several surveys in more recent decades have documented dramatic declines in the frequency of the *carbonaria* morph, which are associated with decreasing air pollution and changes in relative fitness. The most complete data set is that collected by Sir Cyril Clarke for Caldby in north-west England (Clarke *et al.* 1985, 1994; also see Majerus 1998; Cook *et al.* 1999). The decline in *carbonaria* throughout much of urban Britain has been largely matched by an increase in the wild-type, non-melanic *typica* morph. Three sets of data collected in England provided estimates of the fitness of *carbonaria* during this period of declining melanism as being 79–89% that of typicals (Cook *et al.* 1986, 1999).

Industrial melanism has also been described in populations of *B. betularia* from some other regions of Europe (see Lees 1981) and in the North American subspecies *Biston betularia cognataria* (melanic morph *swettaria*) (see Majerus 1998). It is therefore of great interest to compare any parallel changes in melanism in such populations to those occurring in Britain. Comparisons of recent samples of *B. b. cognataria* with those from around 1960

have indicated some similarity between the dynamics of the decline of *carbonaria* in northern England and that of *swettaria* in Michigan, USA (Grant *et al.* 1996, 1998; Cook *et al.* 1999).

As in Britain, in the mid-20th century the central, urban regions of The Netherlands were associated with melanism in numerous species of moth, high air pollution and an absence of epiphytic lichen growth on trees (see Barkman 1969; Kettlewell 1973). The present paper follows up an earlier report (Brakefield 1990) on *B. betularia* in The Netherlands, which indicated a sharp decline in *carbonaria* from around 1970 until 1988. Bakker *et al.* (1987) found an increase in species richness of epiphytic lichens on four species of tree, including limes (*Tilia* spp.), in the same region between 1973 and 1984. This was correlated with a progressive decline in sulphur dioxide that had begun approximately ten years earlier. By the mid-1980s this component of air pollution had declined by *ca.* 90% (see fig. 3 in Brakefield (1990)). We resampled several populations of *B. betularia* in 1999 in order to document whether the decline in melanism had continued and to examine further how frequency changes in *carbonaria* have been accompanied by increases in other morphs. The earlier report had suggested more involvement of *insularia* morphs than in northern England. These intermediate melanic morphs are controlled by three additional alleles at the *carbonaria* gene (Lees & Creed 1977). The *insularia* morphs have apparently remained uncommon during the recent decline of *carbonaria* in northern England (e.g. Cook *et al.* 1999). They may have been historically more abundant in other areas of Britain and northern Europe and *insularia* may play a more prominent role in regions where levels of air pollution have changed more gradually or less markedly (see Kettlewell 1973; Lees 1981; Majerus 1998).

## 2. MATERIAL AND METHODS

Extensive collections of *B. betularia* from 1969 to 1973 from throughout The Netherlands were produced using light traps under the coordination of B. J. Lempke (as reported by Kettlewell 1973). Analysis of this material, largely using Lempke's data, was described by Brakefield (1990). In addition,

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Table 1. Numbers of the five major non-melanic and melanic morphs of the peppered moth *B. betularia* in samples obtained from the indicated localities in 1999

(I1–I3 are the three intermediate *insularia* morphs from pale to dark. Each sample is divided into portions collected early and later in the flight season.)

locality and sample	numbers					total
	<i>typica</i>	I1	I2	I3	<i>carbonaria</i>	
Leiden						
early	34	4	11	7	1	57
late	19	5	8	3	0	35
total	53	9	19	10	1	92
Voorschoten						
early	33	8	8	9	0	58
late	127	20	30	27	2	216
total	160	28	38	36	2	264
Schiedam:						
early	11	2	8	6	0	27
late	27	5	8	14	4	58
total	38	7	16	20	4	85

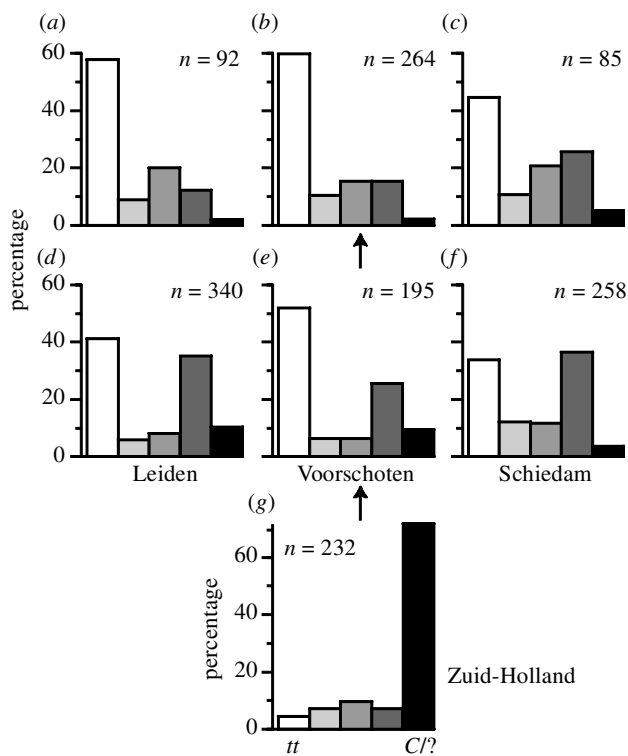


Figure 1. Changes in morph frequencies in *B. betularia* at localities in the province of Zuid-Holland in The Netherlands over the three collecting years: (a–c) 1999, (d–f) 1988 and (g) 1969. The increased degree of shading from left to right represents the five morphs in sequence: *typica* (*tt*), *insularia* 1, *insularia* 2, *insularia* 3 and *carbonaria* (*C*/?).

several large samples of the moth were collected using assembly traps in 1988 in the province of Zuid-Holland near the cities of Leiden, Den Haag (at Voorschoten) and Rotterdam (at Schiedam and Hoogvliet). These samples were compared with the pooled data from Lempke’s survey for all collecting sites within the same province (which had yielded homogeneous samples). The *carbonaria* morph frequency had declined from ca. 70 to 10% over 16–19 generations (Brakefield 1990).

In the present study, we resampled populations in Leiden, near Den Haag (at Voorschoten) and near Rotterdam (at Schiedam) in June 1999. The localities lie approximately along a north to south transect of ca. 30 km in length with Voorschoten less than 10 km south of Leiden. Assembly traps baited with reared female moths were again used for collecting adult males. The trapping sites at Voorschoten were near or at the edge of a large area of woodland, while those at the other two localities were in more urban environments.

Moths were scored as in earlier studies (Brakefield 1990). They were classified into five morphs: black *carbonaria*, three intermediate *insularia* (I3, I2 and I1 from darkest to palest) and non-melanic *typica*. These morph classes follow the dominance hierarchy for the five alleles, with *carbonaria* the top dominant and *typica* the bottom recessive (Kettlewell 1973; Lees & Creed 1977). As in the earlier survey, mistakes in classification were most likely to occur at the boundaries between *carbonaria* and I3 and between I1 and *typica*. However, most moths were in good condition when inspected and the key characteristics involving upper hindwing patterning and dorsal body coloration usually provided little or no uncertainty, even at these boundaries (see Brakefield 1990). The mid-*insularia* (I2) is particularly distinctive.

3. RESULTS

Table 1 gives the morph frequencies for 1999 from the localities in Zuid-Holland and the data for all three surveys are shown graphically in figure 1. *Carbonaria* declined from ca. 70% in 1969–1973 to ca. 10% in 1988 (with a lower frequency at Schiedam near Rotterdam). The decline appears to have continued up to the present day, with only seven out of 441 moths collected in 1999 being scored as *carbonaria*. Table 2 gives the pooled samples for the three surveys in Zuid-Holland and includes estimates for allele and morph frequencies assuming Hardy–Weinberg equilibrium (see Hartl & Clark 1997, pp. 88–92). There is some correspondence between the estimates of the allele frequencies and the observed morph frequencies. In particular, the increased frequency of the darkest *insularia* morph in 1988 was

Table 2. Numbers and morph frequencies for pooled samples of the peppered moth *B. betularia* from the Dutch province of Zuid-Holland in each sampling period together with estimates of allele and morph frequencies assuming Hardy-Weinberg proportions

sample	<i>typica</i>	I1	I2	I3	<i>carbonaria</i>	total
numbers						
1969–1973	10	18	23	18	164	232
1988	331	70	77	259	56	793
1999	251	44	73	66	7	441
allele frequencies						
1969–1973	0.208	0.139	0.122	0.072	0.459	—
1988	0.646	0.065	0.065	0.188	0.036	—
1999	0.754	0.064	0.095	0.079	0.008	—
observed morph frequencies						
1969–1973	0.043	0.078	0.099	0.073	0.707	—
1988	0.417	0.088	0.097	0.327	0.071	—
1999	0.569	0.100	0.166	0.150	0.016	—
expected morph frequencies						
1969–1973	0.043	0.077	0.100	0.073	0.708	—
1988	0.417	0.088	0.096	0.326	0.072	—
1999	0.569	0.101	0.164	0.150	0.016	—

accompanied by a higher allele frequency. The frequency of the *carbonaria* allele declined over the 30 generations of the whole sampling period from *ca.* 0.5 to less than 0.01, while the bottom recessive *typica* allele increased from *ca.* 0.2 to 0.75.

The earlier period of the decline in *carbonaria* was matched by increases in the darkest *insularia* (I3) and in the non-melanic *typica*. The increases were clear for both morph and allele frequencies (table 2). There was little if any response in the other *insularia* morphs (I2 and I1), although the allele frequencies may have declined. In contrast, there was an increase in the mid-*insularia* (I2) morph of up to *ca.* 15–20% during the last decade, which appears to have been accompanied by a small increase in allele frequency (table 2). The increase in *typica* also continued. These changes occurred largely at the expense of the darkest *insularia* (I3). These observations of phenotypic change were supported by heterogeneity  $\chi^2$ -tests performed across years (with or without combining *carbonaria* and I3), although comparisons involving Schiedam only approached significance (see also Brakefield 1990). The temporal changes in morph frequencies were broadly or closely parallel across localities.

The moths were trapped over the whole flight period from 11 June to 16 July in 1999. A marked peak in captures occurred in the second week of July, particularly at Voorschoten. Dividing the captures into an early and a late period (11 June–1 July and 2–16 July) enabled examination of any change in morph frequencies within the season (table 1). No trends were apparent ( $\chi^2$ -test statistics always non-significant whether using four classes with I3 and *carbonaria* combined or *typica* versus all melanics combined).

4. DISCUSSION

*Carbonaria* has been transformed from the most common to the rarest morph in populations in less than 30 generations in central, urban regions of The

Netherlands. The dominant *carbonaria* allele has shown a sharp decline in frequency. The rate of decline is of a similar order to that observed in northern England (Cook *et al.* 1999). However, in comparison with the latter populations, where the *carbonaria* allele is being replaced by the *typica* allele, there is an indication of a more complex dynamics involving changes in morphs specified by other alleles at the *carbonaria* locus in The Netherlands. The frequency of the non-melanic *typica* has increased progressively from around 1970 to the end of the century. Initially, there was also a marked increase in the darkest *insularia* (I3). This phenotype of *insularia* then declined with some reduction in allele frequency, while the paler mid-*insularia* (I2) morph became more abundant, although without such a substantial increase in allele frequency (see table 2).

Thus, the decline in *carbonaria* in urban environments of The Netherlands is being accompanied not only by an increase in *typica* non-melanics, but also by changes in *insularia* intermediates. At least one of the latter changes has involved a period of increased abundance, which was apparently accompanied by a rather sharp increase in allele frequency. Grant *et al.* (1996) found that the frequency of the class of *insularia* morphs increased slightly at Caldby in north-west England over a period of monitoring the decline in the *carbonaria* morph from 1959 to 1993. Our examination of the same extensive data set for the Caldby locality (Clarke *et al.* 1994) showed that the transition there from *carbonaria* to *typica* has occurred without any other changes in allele frequencies (figure 2). Thus, assuming a single *insularia* allele of intermediate dominance (the *insularia* morphs were pooled) and Hardy-Weinberg proportions, the allele frequency remained remarkably constant over this period at around 0.02 (only during the period of very rapid decline in the *carbonaria* morph from 1976 to 1986 were frequencies in several alternating years a little higher). In contrast, over a period of 30 years in The Netherlands, *insularia* morphs have shown an overall increase in frequency whilst the

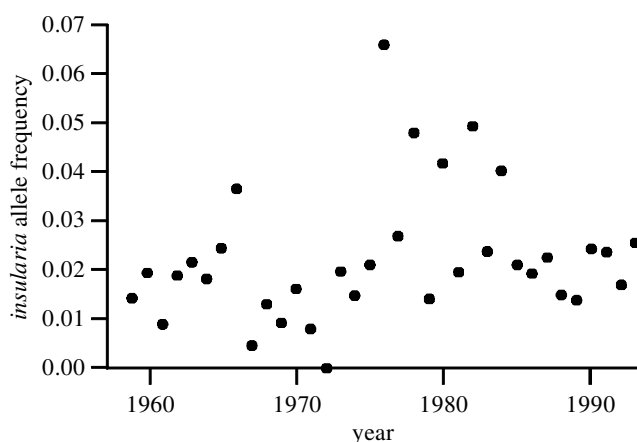


Figure 2. Estimates of the combined frequency of the *insularia* alleles for the Caldy population of *B. betularia* obtained from analysis of the morph frequencies given by Clarke *et al.* (1994) and under the assumption of Hardy–Weinberg proportions. The average sample size per year was 494 moths.

pooled allele frequency has declined by approximately 0.1 (see table 2).

Regular visual inspections of trunks of trees growing in Leiden and its environs over the last decade have shown that the earlier increase in species richness first documented by Bakker *et al.* (1987) has now yielded dramatic changes in the coverage of tree trunks and upper canopy branches by crustose and foliose lichens (for further discussion see Liebert & Brakefield (1987)). For example, the south-west sides of the trunks of extensive 30–50-year-old plantings of lime trees (*Tilia* spp.) in Leiden now consistently have a luxuriant coverage of such lichens, the foliose species of which were only present as scattered, colonizing individuals in 1988 (as noted by Brakefield (1990)). Such changes may now appear dramatic to our eyes and could be thought to influence strongly the relative conspicuousness of the morphs when exposed at rest to birds hunting by sight. However, the potential links between epiphytes and melanism in the peppered moth are not supported by any direct data for a causal relationship and several authors have argued that, in their view, too much emphasis has been given to their role (e.g. Clarke *et al.* 1994; Grant *et al.* 1996, 1998; see also the discussion in Cook (2000)).

The history of air pollution in The Netherlands has differed from that in many regions of Britain due to a comparative absence of particulate pollutants. It has been dominated by gaseous pollutants, in particular sulphur dioxide (see Brakefield 1990). This historical feature of the environment, perhaps through interactions between the resting behaviour of adult moths and the dynamics of epiphytic communities on trees, may have played a role in the differing details of the dynamics of the decline in the *carbonaria* allele in the latter part of the 20th century. Regardless and in contrast to northern England, *insularia* morphs were already present at substantial frequencies as the decline in *carbonaria* took shape in The Netherlands. Natural selection is possibly more tightly associated with gradual changes in resting background and visual predation in The Netherlands giving rise to a more graded response in terms of frequency changes in the morphs. However, a more

straightforward explanation may be involved since, if the *insularia* morphs have a sequence of intermediate relative fitnesses, successive rises and falls would be expected in their frequencies even if their fitness values were constant in time during the decline (L. M. Cook, personal communication after examination of simulation data). Such a scenario does not necessarily involve any complex dynamics, for example in terms of successive shifts in selection gradients. Whatever the precise details of the biological reasons why spatial variation and temporal change in relative fitness occur in *B. betularia*, the documented declines in melanism provide fascinating examples of dynamic evolution.

We dedicate this paper to Sir Cyril Clarke FRS who contributed substantially to the understanding of industrial melanism and who did much to stimulate our own interests in the Lepidoptera. We are very grateful to Dr L. M. Cook and Dr B. Grant for their most constructive comments on the manuscript. We also thank M. Brittnij for preparing the figures.

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